Recent Development and Perspective of Agricultural Biotechnology in Taiwan

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Taiwan is an island which covers an area of 13,814 square miles with a high density population of 21 million and poor natural resources.In the past 15 years, its economy has moved rapidly from an initial agricultural base into an industrial society. Recently, under the trends of industrialization, free trade, and international development, Taiwan's agriculture has faced increasing pressure from international competition and production efficiency. The Council of Agriculture (COA) realized that biotechnology would play an important role in promoting agricultural productivity and development, and for this the COA initiated a national research program starting in 1987 to support and assist in many biotechnological research projects which have been conducted extensively in previous years in various research institutions and university laboratories. Due to limited manpower and resources, this program has research projects prioritized, screened and evaluated to ensure the quality and integration of the research project. A brief review in trends of research and development of agricultural biotechnology in Taiwan is presented in the followings.

I. Direction

The following research topics have been targeted in the COA's Agricultural Biotechnology Program:

1. Diagnosis and control of plant diseases and pests

Damages from plant diseases and pests are major limiting factors for crop production in Taiwan. Accurate diagnosis of crop diseases and pests not only helps control but can also prevent their spread. Serological kits and DNA probes are the mainfocus for the development of detection and diagnostic technology. Isolation and transfer of disease resistant genes and development of recombinant viruses against pests are emphasized for disease and pest control.

2. Plant gene transfer technology

This research project is a very important area in agricultural biotechnology. Once an

useful gene is cloned, it can be transferred into crops to make genetic improvements. This project particularly emphasizes genes related to disease-resistance, insect -resistance, flower color, cold-resistance, protein content and high photosynthesis rate.

3. Propagation and improvement of agronomic and horticultural crops

The principles of cell totipotency express that plant tissue, somatic cells or sex cells are capable of regeneration through organogenesis or embryogenesis and transformation into morphological bipolarity. The development of *in vitro* manipulation of morphological transformation has made a great contribution in plant science and agricultural industry. Additionally, the *in vitro* culture technique is also a prerequisite for the development of genetic transformation through plant cells or calli.

4. Propagation of forest crops and production of secondary metabolites

Four indigenous forest crops in Taiwan, viz. *Cinnamomum kanehirae, Taxus mairei, Chamaecyparis* spp., and *Picea morrisonicola,* are studied in this project. The research focuses on using tissue cultures to propagate and rejuvenate superior aged trees for *C. kanehirae* and investigate the taxane contents in the needles of individual tree and to produce taxol for *T. mairei.* In addition, for *Chamaecyparis* spp. this project tends to reproduce artificial plantlets from in vitro shoots or somatic embryos and to establish an in vitro system via somatic embryogenesis for *P. morrisonicola.*

5. Food biotechnology

The main goal of food biotechnology research and development is to increase the competitiveness of food industries by studying the cloning and expression of food related enzymes and ingredients as well as the improvement of expression of vector-host systems.

6. Aquaculture

The application of biotechnology in aquaculture has been categorized into four topics according to human resources as well as research interests in Taiwan, which cover: (a) Application of biotechnology to enhance fish adapatation to the environment; (b) The development of transgenic aquacultural animals; (c) Cryopreservation of embryo of finfish and shellfish; (d) The development of diagnostic kits for shrimp diseases and development and evaluation of vaccines for shrimp and fish diseases.

7. Animal and veterinary science

Biotechnology offers tremendous potential for improving animal production. Research areas of higher priorities for the sake of solving present problems and effectively using resources include: (a) the development of diagnostic kits for animal diseases and for food safety inspection; (b) recombinant vaccines for the prevention of economically important animal diseases; (c) embryo transfer and genetic modification of domestic animals; and (d) engineering of microorganisms for reducing the pollution caused by animal waste.

II. Current Status and Major Achievements

1. Diagnosis and control of plant diseases and pests

The problems of crop damages caused by plant diseases and pests have been increasing in recent years in Taiwan. In order to diagnose the causes of crop damages accurately and quickly so that remedial measures may be taken promptly, biotechnology is employed to develop monoclonal antibodies and DNA probes for the detection and identification of plant disease causal agents. It is also used to develop measures for controlling diseases and pests. Presently, several monoclonal antibodies were obtained to detect viruses such as bamboo mosaic virus, banana bunchy top virus, and to detect phytoplasmas of peanut witches* broom and sweetpotato witches* broom. The ELISA techniques were applied to field survey and detection, establishment of healthy seed/seedling programs and detection of mild strain viruses for cross protection. In addition, DNA probes were also acquired for plant pathogen detection as well as to increase sensitivity of disease diagnosis. Identification of smut fungi and detection of *Pythium* spp. in hydroponic culture are two examples.

2. Plant gene transfer technology

There are 4 subtopics under this title:

(A) Transformation of disease-resistant genes in horticultural and agronomic crops

Agrobacterium-mediated transformation system of tomato, pepper, sweet melon, water melon, cucumber, and papaya has been established primarily using GUS reporter and disease-resistant genes. Three transgenic lines of papaya containing PRSV coat proteins (CP) were screened and showed different levels of resistance to the virus. Peppers transformed with a truncated cucumber mosaic virus (CMV) showed either no signs of CMV infection or showed milder symptoms than untransformed plants. In addition, Bt insecticidal crystal protein genes were transferred into cauliflower, cabbage, broccoli and Chinese cabbage via *Agrobacterium* spp.

(B) Quality improvement of *Phalaenopsis* cut flower utilizing biotechnology

An ACC synthase gene from *Phalaenopsis* has been isolated and sequenced. Plasmids for gene transfer containing the sense and antisense ACC synthase sequence have been constructed as well.

(C) Construction of linkage map based on RAPD and RFLP markers in sweet melon

Constructing a genetic linkage map for sweet melon is in progress using RAPD, RFLP, and isozyme markers. Two hundred and twelve mapped cabbage, tomato and tobacco clones against sweet melon genomic DNA have been probed to establish STSs.

(D) Resistance breeding of transgenic banana against bunchy top virus (BBTV)

One clone of replicase gene from banana bunchy top virus was sequenced and constructed into a plant gene expression vector in antisense orientation.

3. Propagation and improvement of crops

(A) Massive production of quality nurseries

The *in vitro* technology of plant tissue culture has diverse advantages stimulating the progress in plant propagation and improvement. It has become routine technology in the massive production of quality nurseries. It is particularly useful in vegetatively propagated crops such as banana, papaya, passion fruit, citrus, potato, strawberry, orchids. It is worth mentioning that the micropropagation of banana is already extended to most producing countries. Two million banana plantlets can be produced in one crop season in Taiwan and 16 million total each year, which is so far the best record in the world.

(B) Production of artificial seeds or F_1 hybrid clones

Recently, the factors in the determination of embryogenic cells are much understandable and manipulative. The suspension of embryogenic cells destining to somatic embryogenesis could be homogeneously induced in banana and papaya, obtaining a high rate of somatic embryo plantlet conversion. However, the induction of physiological dormancy and encapsulating materials are critical to the development of artificial seeds. It is expected that the development of a high efficiency cell and somatic embryo culture system would be potential in production of artificial seeds or Flhybrid clones. DNA fingerprinting has been proven a powerful tool in assaying somaclonal variation.

(C) Establishment of in vitro conservation system

The *in vitro* culture is also useful in rescuing rare and endangered species. The *in vitro* culture also performs as an efficient technique for genetic resource conservation. It is particularly important for the establishment of a clonal germplasm bank in vegetatively propagated crops. Hundreds of species are preserved in minimum growth storage.

(D) Application of *in vitro* breeding

The applications of *in vitro* breeding in either sexual or asexual crops have made some progress. The combinations of somaclonal variation, in vitro mutagenesis, and selection pressures greatly increase opportunity for obtaining desirable mutants, suchas disease resistance achieved in strawberry and *Cavendish* banana. The anther culture of *Brassica* has regenerated haploid plants. The *in vitro* technique is also very useful in overcoming sexual barriers such as incompatibility.

4. Food biotechnology

(A) Development of the novel amylolytic yeast used in food industry

Due to its rapid growth rate and high ethanol tolerance, brewing yeast *Saccharamyces cerevisiae* has been widely used in alcohol, wine and soy sauce manufacturing. However, the absence of starch digestion ability greatly limits its application. A gene encoding the isoamylase was cloned from *Pseudomonas* spp. by PCR.

To further hydrolyze linear starch (amylose) to glucose to facilitate yeast fermentation, a glucoamylase gene was also cloned from *Aspergillus* spp. This gene was then site-specifically integrated into the *S. cerevisiae* chromosome by integration plasmid with the help of homologous ribosomal DNA. This specially designed yeast showed great potential in light beer and soybean sauce manufacturing.

(B) Production of natural pigment by plant bioreactor culture system

There is a large demand for natural red and yellow pigments on the domestic market. We therefore intend to develop plant bioreactor culture systems to lower the cost.Sterile callus of red beet and purple carrot were established. Optimal medium composition and bioreactor operating conditions were investigated to promote the pigment synthesis of purple carrots and red beets.

(C) Enhancing the utilization of food waste by genetic engineering methods

Cellulose is by far the most abundant carbohydrate available from plant biomass. *In vitro* reconstitution of cellulosome from two recombinant components is under study. The accomplishments of this work shall greatly benefit cellulose degradation research and applications.

Utilization of chitin, another important food processing waste, is also under study. A strong chitin degradation bacterium was isolated from a local sea food identified as *Aeromonas hydrophila.* A 4.5kb corresponding chitinase gene was cloned and expressed in Escherichia coli. Not only can chitinase be used in food waste recycling, but also it has many potential applications in pharmaceutical ingredients and functional foods production.

5. Propagation of forest trees and production of secondary metabolites

Cinnamomum which is an indigenous forest tree. For *C. kanehirae* clonal propagation and somatic embryogenesis have been successfully established *in vitro* by using plants derived from rootsprouts and seeds. For *T. mairei* selection of superior trees containing high taxanes in their needles, embryo-derived plants *in vitro,* and the cultures of callus and cell suspension have been achieved.

6. Aquaculture

(A) Application of biotechnology to enhance the adaptation of fish to the environment

When the cold front passes each year, a large number of cultured fishes die, resulting in losses of hundreds of millions of NT dollars. This project proposes to use biotechnology to study the tolerance of fish under cold stress and to learn the mechanisms for avoiding losses in the future.

The cold tolerance of milkfish was enhanced, and the possibility of the addition of highly unsaturated fatty acid and antifreeze protein as feed additive to improve the cold tolerance of milkfish was evaluated.

(B) Development of the transgenic finfish and shellfish

For gene transfer techniques, a feasibility study on transferring growth hormone genes into finfish, scallop and shellfish has been conducted.

(C) Cryopreservation of the embryos of finfish and shellfish

Studying factors affecting the cryopreservation of the embryos of finfish, shrimp and shellfish may present the opportunity to understand and solve the problem. The tol-

erance of the embryos and the fertilized eggs to antifreezers and its osmotic adjustment is a subject of interest.

(D) Development and evaluation of the diagnostic kits for shrimp diseases

MBV and WSBV are common viruses causing diseases in the artificial propagation of young shrimp. It is therefore an urgent need to investigate the early infection of MBV or WSBV in order to control or treat these diseases.

(E) Development and evaluation of the vaccines for shrimp and fish diseases

The vaccine for eel is made from a mixture of dual bacterial pathogens. This project is aimed at understanding the relationship among the bacteria dosage, frequency of treatments and antibody titer of the oral or immersed vaccines. The major bacterial pathogens of shrimp were also developed into a vaccine to combat shrimp diseases in the hope of reducing the death rate during the artificial propagation.

7. Animal and veterinary science

(A) Diagnostic methods and the development of related diagnostics

The monoclonal antibodies against economically important diseases such as Newcastledisease, pseudorabies, hog cholera, and actinobacillus pleuropneumonia, were produced and/or collected for developing the diagnostic kits. The ELIZA diagnostic kit for detecting the residue of sulfamethazine, antibiotics for pork, was also developed.

(B) Vaccine development by genetic engineering

Research has continued to develop recombinant vaccines such as pseudorabies genedeleted vaccine and live vector vaccines for pseudorabies.

(C) Animal breeding

Various biotechnologies including artificial insemination, super-ovulation, estrous synchronization, embryo transplantation, in vitro maturation and fertilization, embryo pitting and sex determination of split embryos, nuclear transplant, embryo cloning, and injection of genes into newly fertilized eggs have been well established and applied for years. Some real benefits have also been gained, especially with the embryo transfer of cattle.

III. Perspectives

1. Diagnosis and control of plant diseases and pests

Researches will be continued to produce monoclonal antibodies and DNA probes for early detection of plant pathogens and diagnosis of plant diseases with accuracy and promptness. Development of diagnostic techniques will also be conducted to prevent the spread of plant disease and to assist in the production of pathogen-free healthy seeds/seedlings. To develop plant disease and pest control measures, researches will be devoted and expanded to identify resistant genes to produce resistant transgenic plant varieties and to develop microbial control agents.

2. Plant gene transfer technology

Despite strong research programs, commercial utilization of research results are necessary for the development of the biotech industry. Creation of more productive varieties of agriculture and horticulture crops which could be utilized on a commercial scale is the ultimate achievement in the development of biotech.

Likewise, the transformation of research techniques, useful products or newly developed cultivars from research institutes to farmers or producers is also important. All the products from upstream research must be market-oriented and will have to be applicable in a less sophisticated scientific environment, as well as in mass production in order to be transferable as soon as possible to the applied level.

3. Propagation and improvement of horticultural crops

We still have some limited knowledge regarding genetic control of yield or quality. However the highlight of in vitro manipulation in cell genetic transformations has become feasible. The regenerative capability of plant cells should be inducible in the most major crops, not just focus on the model plants. The efficient cell and somatic embryo conversion system would be of benefit for engineering cells. The deliveries of foreign genes into embryogenic cells through various vectors have progressed in banana and Brassica. The regenerants were assessed to be transgenic plants.

4. Food biotechnology

In addition to the gene recombination and tissue culture research mentioned above, industrialization of microbial production of food product quality will also be emphasized in the food biotechnology sector in the future.

5. Propagation of forest crops and production of secondary metabolites

For *C. kanehirae*, rejuvenation of old superior trees for clonal propagation is economi-

cally important. For *T. mairei,* both selection of individual trees with needles containing high taxol and 10-deacety baccatin III and production of taxol from selected cell lines are economically feasible. For *Chamaecyparis* spp., clonal reproduction using artificial seeds is a time- and money-saving method in comparison to the traditional method via cutting. For *P. morrisonicola,* establishing a system of somatic embryogenesis is a useful technique for large scale propagation.

6. Aquaculture

The value of aquacultrue in our country has comprised a large share of total fishery output. However, due to an increasing pollution problem, epidemics of shrimp diseases, and so forth, have caused severe difficulties for further development of aquacultrue in Taiwan. Fortunately the rapid development of biotechnology may be applicable in aquaculture to provide space for development, for example: breeding improvement, improving the cultural environment, disease diagnosis, and the development of vaccines of high potency.

7. Animal and veterinary science

The following research and development areas have higher priorities for the sake of solving present animal industry problems and effectively using resources.

(a)Development of diagnostic kits for animal diseases.

(b)Development of diagnostic kits for food safety inspection.

(c)Development of animal vaccines and their related adjuvant and assay technologies for the retention of economically important animal diseases.

(d)Embryo transfer and genetic modification of food producing animals.

(e)Engineering of microbes for reducing the pollution caused by animal waste.

8. Biopesticides and biofertilizers

In addition to those mentioned above, the Council of Agriculture has just initiated a research program to develop biopesticides and biofertilizers.

Biopesticides as natural pest control agents are valuable because they are environmen -tally friendly and target-specific. They can be used to partially replace toxic chemical pesticides presently applied extensively in the field. For the time being, development of bioinsecticides and biofungicide is targeted.

Biofertilizers are beneficial microorganisms which can facilitate the uptake of nutrients by crops, and help cutting down the need for chemical fertilizers. The major emphasis is to improve the capability of some potentially useful biofertilizers, such as nitrogen-fixing microbes, mycorrhizal fungi, phosphorous-solubilizing microbes, and

thermophilic cellulose-decomposing microbes. The ultimate goal of the researches is to improve microbes to have composite functional mechanisms in facilitating nutrient uptake.

In order to promote the application of biotechnology, a plan called "Bio-technology Upgrading Program" was issued by the Executive Yuan, ROC. In the area of agriculture, the plan has prioritized flower, animal vaccine, and biopesticide for development. It is expected that related biotechnology industry will be established in a 3-5 year time table. To implement the plan, the Council of Agriculture (COA) has set up three working groups, one for each category, and held meetings with people from research and industry sectors to discuss on how to meet the needs of and to provide technical assistance to the industry for commercial manufacture of biotech products. The followings describe current development of the three categories:

I. Flower Industry

In Taiwan, the application of biotechnology on modern flower industry is mainly focused on the micro-propagation for mass production of healthy young plants or seedlings by the use of tissue culture technique. To use the technique of gene transformation for creating new varieties is still on the stage of research, and mainly performed by government supported institutes. In micro-propagation, owing to the general know-how is easy to acquire and the facilities are easy to install, quite a few nurserymen have been engaged in this field. Today there are more than 200 producers in total, however, only less than ten are performing large-scale production. Most producers are running their business on a small-scale level, and are of part time or family companies. Among the nursery business in all flower crops, Phalaenopsis, with self-developed varieties and leading technique, has become the most important kind. There are millions of seedlings in Phalaenopsis produced for export every year. The next in order are cymbidium, gerbera, anthurium, oncidium, cypripedium, and some foliage plants. Recently, several kinds of bulb flowers (bulbs or tubers) such as lily, calla, gladiolus, Lycoris, and amaryllis have also been put a high premium on R&D. A Technical Service Team, composed by the expert in different fields including biotechnology, floriculture, agricultural engineering, plant pathology, and postharvest, has been organized by COA since 1996. Its goal is to help nurserymen to overcome their technique bottleneck and to lead investors to a better business management. The service is focused on the orchids and bulb flowers, in order to produce high quality and high price seedlings. To help managing tissue culture product, a computer operation system has been successfully set up by the

service team, and its technique has been passed over to the related producer.

Right now the emphasized items for industrial development are as following:

1. The breeding and extension of the newly developed flower crops, in order to create self-developed varieties which are very crucial for the prosperity in the flower industry.
2. The establishment of automation system in bulb flowers propagation, in order to produce healthy bulbs in large scale and reduce the production cost.
3. The qualification and registration for orchid variety and seedlings, and the improvement of mass propagation technique for the development of orchid nursery industry.
4. To assist and guide flower nursery companies to enhance R&D and to breed new varieties, in order to produce healthy and true-to-the-type seedlings, and to establish a well organized flower industry for international marketing.

II. Vaccine Industry for Animal use

Animal vaccines play very important roles on animal disease control and the development of animal industry. It is a technique intensive industry, and every country in the world is paying more and more efforts for its development.

Most of the animal vaccines used in Taiwan are imported from foreign countries. The market share of local made vaccines is only about 25%. In Taiwan, there are 8 animal vaccine manufactures, and all of their production scales are so small. Since all of them are lacking of abilities for the development of vaccines, and their research resources are only from universities or research institutions, the vaccines they can produce are from traditional technologies. Due to the insufficient abilities for the development of new products and the improvement of quality, the difference between Taiwan's and imported animal vaccine value and quality becomes greater and more greater. Consequently, the market share of the local made vaccines decreases very rapidly.

In recent years, animal vaccines production has become a great potential technology, and the market share of vaccines in the Asia Region has also become potent. How to encourage and assist our animal vaccine industry on the improvement of research abilitie s and quality levels, especially on the production of the vaccines for specific diseases which are not endemic in other countries and have great market share potential ones, is an emergent task for us to reduce the influence of being a WTO member countries. The key items fot the development of this industry are:

1. Vaccines produced by the traditional technologies:

   These products include inactive vaccines for controlling Pleuropneumonia as well as Actinomyces and specific disease infections; Bordetella, Pastrella, Actinomyces, Salmonella and Pseudorabies multivalent inactive vaccine; swine Parvo virus inactive vaccine; Hog cholera and Erysypella bivalent live vaccine; Hog cholera and Japanese encephalomylities bivalent live vaccine; Porcine Reproductive and Respiratory Syndrome live vaccine; Infectious Brochities live vaccine; Newcastle disease, Infectious Bursal disease, Egg drop syndrome, Infectious bronchities, coryza and Escherichia coli multivalent inactive vaccine.

2. Vaccines produced by new biotechnologies:

   These products include gene deletion pseudorabies live and inactive vaccines.

3. The improvement on the preparation of adjuvant:

   Adjuvant is the most important ingredient for animal vaccines. Since they are strongly related to the quality and the efficacy of vaccines, more attentions should be paid to their development.

III. Biopesticide Industry

Damages caused by diseases and insect pests is one of the limiting factors in agricultural production in Taiwan. Due to the rapid increase of importation of agricultural products and development of horticultural industry, the incidence of new diseases and insect pests have occurred. To reduce the damages, pesticide application has been adopted as major control method. This has resulted in great concern about pesticide residue and environmental pollution. The current policy in plant protection is to develop accurate diagnostic technology for proper control of diseases and insect pests, and to explore non-pesticidal control measures. Developing biopesticides to partly replace the uses of pesticides is a worldwide trend in plant protection. Biotechnology has provided a powerful tool for that purpose. In order to protect crops, The COA has granted researches to develop antibodies and nucleic acid probes for fast and accurate detection of disease causal agents, and to employ genetic engineering technology to develop gene transferred plants with resistance or vaccines for cross protection.

Presently the aims of biopesticide development are to improve methods for screening organisms with high protection efficiency, for instance, nematodes and microorganisms including viruses, fungi, and bacteria, as well as to develop technology and formula for mass propagation and fermentative production in order for further establishment of processes for

commercial manufacture. This will facilitate the efficient use of biopesticides in Taiwan. The major points of current development as follows:

1. Collecting and improving local *Bacillus thuringiensis* (Bt), establishing technology for mass propogation, and developing Bt insecticide.

2. Improving methods to screen insect-viruses, entomopathogenic fungi, nematodes and developing fermentative production models for bioinsecticide development. The target organisms include nuclear polyhodrosis virus, *Steinernema* spp., *Beauveria bassiana, Metarhizium anisopliae,* and *Nomuraeu rileyi,* etc.

3. Screening elite virus strains and antigonistic microorganisms from soils or leaves, for example, *Trichoderma* spp. and fluorescent pseudomonads, establishing fermentative production models and formula, and developing biofungicides for disease control.